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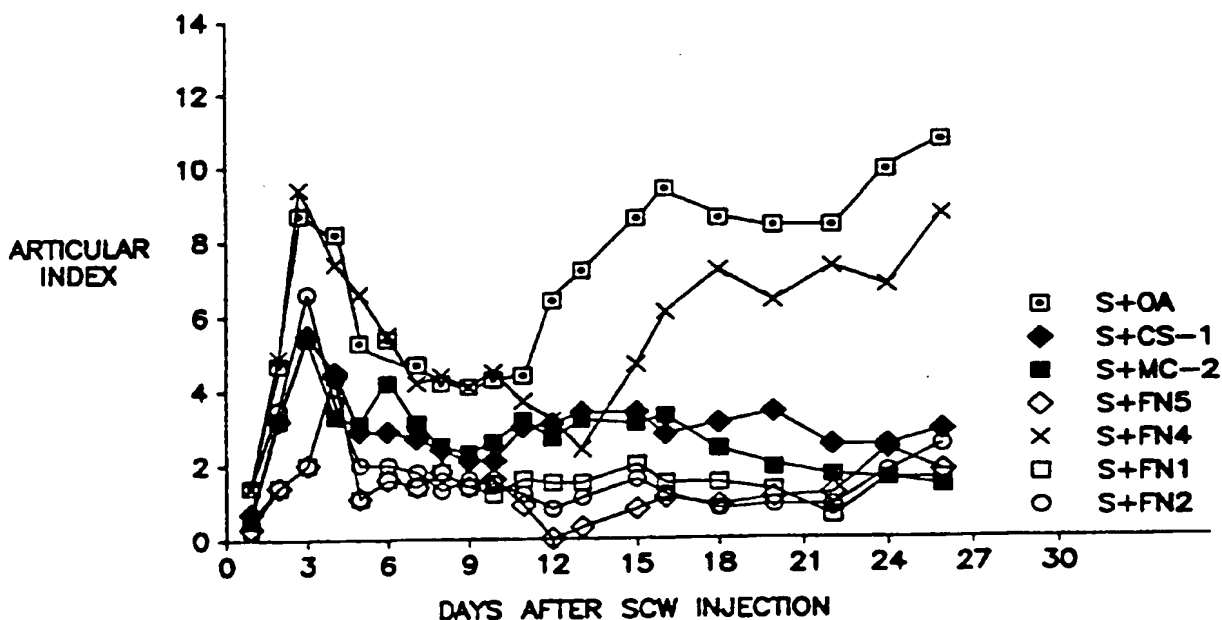
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(54) Title: POLYPEPTIDES USEFUL FOR TREATING INFLAMMATORY DISORDERS



(57) Abstract

A method for treating acute or chronic inflammatory or autoimmune disorders using polypeptides with fibronectin or related activity is provided. The method involves administering an effective amount of one or more polypeptides corresponding to isolated amino acid residue sequences of the 33 kD carboxy terminal heparin-binding region located on the A chain of fibronectin or an RGD-containing amino acid sequence within the 11.5 kD RGD-mediated cell adhesion region located on all isoforms of fibronectin to effectively suppress inflammation and the accompanying impairment of tissue function in a patient.

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POLYPEPTIDES USEFUL FOR TREATING INFLAMMATORY DISORDERSBACKGROUND OF THE INVENTION

The adhesion of mammalian cells to the
5 extracellular matrix is of fundamental importance in
regulating growth, adhesion, motility and the
development of proper cellular phenotype. This has
implications for normal development, wound healing,
immunity, chronic inflammatory diseases, and tumor
10 metastasis. Evidence accumulated over the last several
years suggests that the molecular basis for the adhesion
of both normal and transformed cells is complex and
probably involves several distinct cell surface
molecules. Extracellular matrices consist of three
15 types of macromolecules: collagens, proteoglycans and
noncollagenous glycoproteins. The extracellular matrix
molecule which has been most intensively studied with
regard to cell adhesion is the noncollagenous cell
adhesion glycoprotein, fibronectin, which is present in
20 plasma, cell matrices, basal lamina and on cell
surfaces. The fibronectin from plasma consists of a
disulfide-bonded dimer having a molecular weight of
450,000 daltons. The two subunit chains ("A" and "B"),
each of about 220,000 daltons, are observed under
25 reducing conditions. This form of fibronectin will be
referred to as "fibronectin" hereinafter.

Polypeptides from a 33 kD carboxyl terminal
heparin-binding fragment of the A subunit fibronectin
which promote adhesion and spreading of endothelial
30 cells and melanoma cells are described in United State
Patent Nos. 4,839,464 and 5,019,646. The synthetic
polypeptides corresponding to fibronectin residues
described in these patents are disclosed as useful to
(a) assist in nerve regeneration, (b) promote wound
35 healing and implant acceptance, (c) promote cellular
attachment to culture substrata, and (d) inhibit
metastasis of malignant cells.

Evolution of inflammatory and immune reactions
is dependent upon the recruitment and migration of

circulating leukocytes to sites of injury or antigen deposition. The accumulation of leukocytes is dependent not only on chemotactic signals emanating from the inflammatory site, but also on cell-cell and cell-matrix interactions. Many of these cellular and matrix interactions are dependent upon expression of cell surface adhesion molecules (CAMs) [integrins, cell surface proteoglycans, selectins, etc.] which facilitate targeting and retention of circulating cells to sites of immunologic challenge [T. Springer, Nature, 346: 425-434 (1990); S. M. Albeda et al., FASEB J., 4: 2668-2680 (1990); Ruoslahti, J. Clin. Invest., 87: 1-5 (1991)].

Integrins represent a family of cell surface $\alpha\beta$ heterodimeric proteins that mediate cell adhesion to other cells and to extracellular matrix constituents, including fibronectin. Although the role of integrins and other CAMs in mediating arrest and adhesion of inflammatory cells prior to extravasation is complex and poorly understood, emerging evidence suggests that integrins may be pivotal in these events. Therefore, a need exists for a method employing an agent that inhibits or modulates emigration of circulating cells to the site of immunologic challenge as a mechanism to regulate inflammation and its associated disorders.

SUMMARY OF THE INVENTION

The present invention provides a method for treating a number of disease states such as, for example, conditions associated with inflammatory disorders by administering to the patient an effective amount of compositions containing a polypeptide having a sequence of at least about three amino acids corresponding substantially to an amino acid sequence within the 33 kD carboxyl terminal heparin-binding region located on the A chain of fibronectin or an RGD-containing amino acid sequence within the 11.5 kD RGDS-mediated cell adhesion region located on all isoforms of

fibronectin. Preferably, the method involves administering an effective amount of a polypeptide or mixture of polypeptides having the formula:

tyr-glu-lys-pro-gly-ser-pro-pro-arg-glu-val-val-pro-
5 arg-pro-arg-pro-gly-val (I) [SEQ ID NO:1],
lys-asn-asn-gln-lys-ser-glu-pro-leu-ile-gly-arg-lys-
lys-thr (II) [SEQ ID NO:2],
trp-gln-pro-pro-arg-ala-arg-ile (V) [SEQ ID NO:3],
asp-glu-leu-pro-gln-leu-val-thr-leu-pro-his-pro-asn-
10 leu-his-gly-pro-glu-ile-leu-asp-val-pro-ser-thr (CS-1)
[SEQ ID NO:4], and
ser-pro-pro-arg-arg-ala-arg-val-thr (IV) [SEQ ID
NO:5].

Polypeptide I formula represents isolated
15 fibronectin residues 1906-1924. Polypeptide II
represents isolated fibronectin residues 1946-1960.
Polypeptide IV represents isolated fibronectin residues
1784-1792. Polypeptide V represents isolated
fibronectin residues 1892-1899. Polypeptide CS-1
20 corresponds to residues 1961-1985.

Another useful polypeptide in the method of the present invention is:

ile-thr-val-tyr-ala-val-thr-gly-arg-gly-asp-ser-pro-
ala-ser-ser-lys-pro-ile-ser (MC-2) [SEQ ID NO:6]. This
25 polypeptide corresponds to residues 1485-1504. With the
exception of CS-1, all other peptides are common to all
isoforms of fibronectin.

A preferred embodiment of the present invention employs multivalent polypeptide and carrier compound
30 conjugates. For example, conjugates having at least 3
and preferably 4 to 8 polypeptide fragments covalently
bound to a carrier compound such as ovalbumin (OA),
human serum albumin (HSA), other proteins, and
polyethylene glycol (PEG), are useful in the present
35 invention.

According to the present invention, a polypeptide or mixture of polypeptides corresponding to

an isolated region of fibronectin residues is employed to suppress inflammation and tissue destruction.

The present invention also includes a method of treating arthritis or an autoimmune disorder which
5 includes administering a polypeptide or mixture of polypeptides corresponding to an isolated region of fibronectin residues.

The described method can be employed to treat acute inflammatory and immunological disorders and is
10 particularly well-suited to treat chronic inflammatory disorders and/or immune mediated disorders, including autoimmune disorders. Since it is expected that further digestion/hydrolysis of polypeptides from the 33 kD carboxyl portion of the A chain of fibronectin will
15 yield fragments of substantially equivalent bioactivity, lower molecular weight polypeptides corresponding to isolated residues of the A chain of fibronectin are considered to be within the scope of the present invention. While the method described herein utilizes
20 fibronectin polypeptides I, II, IV, V, CS-1 and MC-2, it is to be understood that polypeptides having shorter sequences of amino acids, as well as other polypeptides corresponding to regions within the A and/or B chains of fibronectin with functionally active sequences, are
25 within the scope of the invention. For example, polypeptides having sequences of about 3 amino acids or larger with functionally active sequences are within the scope of the invention. Examples of such short fragment polypeptides include arg-gly-asp-ser (RGDS) and other
30 RGD-containing tetramers. It is known that trimers such as RGD when connected to other amino acids or amino acid sequences are functionally active (e.g., the Ser of RGDS may be substituted to form a functionally active tetramer). Other examples of functionally active small
35 polypeptides within the scope of the invention include ala-arg-ile (ARI), arg-ala-arg-ile (RARI) and other short ARI-containing sequences.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the effect of FN fragments on SCW-induced arthritis. OA-coupled FN fragments were administered i.v. on days 0-4 to SCW-injected rats. Controls included rats given SCW and OA only. Articular indices were determined at indicated intervals (N=3-4/group).

Figure 2 shows the effect of FN fragments on chronic synovitis. OA-coupled FN fragments were administered i.v. on days 11-15 to SCW-injected rats. Control SCW-injected rats received OA. Articular indices were determined at indicated intervals (N=3-4/group).

Figure 3 shows the effect of uncoupled FN fragments on SCW-induced arthritis. FN fragments were administered i.v. on days 0-4 to SCW-injected rats. Controls included rats injected with SCW only. Articular indices were determined at indicated intervals (n=1-3/group).

Figure 4 shows the effect of fibronectin fragments on the weight of mice made genetically deficient in TGF- β 1 (-/-) (this creates a spectrum of symptomology in affected animals). A mixture of fibronectin fragments was administered for 14-18 days intraperitoneally starting 8 days after birth. Controls included untreated TGF- β 1-deficient mice (-/-) and normal wild-type TGF- β 1 (+/+) mice.

Figure 5 shows the effect of fibronectin fragments on the infiltration of inflammatory cells into heart tissue of TGF- β 1-deficient mice.

Figure 6 shows the effect of fibronectin fragments on the infiltration of inflammatory cells into lung tissue of TGF- β 1-deficient mice.

DETAILED DESCRIPTION OF THE INVENTIONStructure of Fibronectin

The structure of fibronectin has been previously described in United States Patent Nos. 4,839,464 and 5,019,646, the disclosures of which are incorporated by reference herein. The A chain digest contains a 11.5 kD RGDS-mediated cell adhesion fragment (domain IV), a 33 kD fragment (domain V) and a 31 kD fragment (domain VI). The polypeptides useful for the present invention correspond to isolated regions of domains IV and V, which are common to all isoforms of fibronectin.

Domain IV is a 11.5 kD polypeptide of 108 amino acid residues corresponding to residues 1410-1517 of all isoforms of fibronectin. Domain IV of fibronectin has been previously described in McCarthy et al., J. Cell Bio., 102, 179-188 (1986), the disclosure of which is incorporated by reference herein.

Synthesis of Polypeptides

Exemplary polypeptides employed in the method of the present invention are described in the above-referenced 4,839,464 and 5,019,646 patents.

The significant chemical properties of peptides useful in the present invention are summarized in Table I, below:

TABLE I

Peptide Charge	Residue Nos.	Hydropathy	Net
		Index	
I [SEQ ID NO:1]	1906-1924	-24.3	+2
II [SEQ ID NO:2]	1946-1963	-32.5	+2
CS-1 [SEQ ID NO:4]	1961-1985	-9.9	-4
IV [SEQ ID NO:5]	1784-1792	-12.2	+3
V [SEQ ID NO:3]	1892-1899	-10.8	+2
MC-2 [SEQ ID NO:6]	1485-1504	-0.8	-1

The polypeptides used in the invention were synthesized using the Merrifield solid phase method.

This is the method most commonly used for peptide synthesis, and it is extensively described by J. M. Stewart and J. D. Young in *Solid Phase Peptide Synthesis*, Pierce Chemical Company, pub., Rockford, IL (2d ed., 1984), the disclosure of which is incorporated by reference herein. This method of synthesis is understood to be illustrative only and not intended to limit the scope of the present invention in any way.

The Merrifield system of peptide synthesis uses a 1% cross-linked polystyrene resin functionalized with benzyl chloride groups. The halogens, when reacted with the salt of a protected amino acid, will form an ester, linking it covalently to the resin. The benzyloxy-carbonyl (BOC) group is used to protect the free amino group of the amino acid. This protecting group is removed with 25% trifluoroacetic acid (TCA) in dichloromethane (DCM). The newly exposed amino group is converted to the free base by 10% triethylamine (TEA) in DCM. The next BOC-protected amino acid is then coupled to the free amine of the previous amino acid by the use of dicyclohexylcarbodiimide (DCC). Side chain functional groups of the amino acids are protected during synthesis by TFA stable benzyl derivatives. All of these peptides of the present invention were synthesized at a University of Minnesota microchemical facility by the use of a Beckman System 990 peptide synthesizer or Applied Biosystems synthesizer.

Following synthesis of a blocked polypeptide on the resin, the polypeptide resin is treated with anhydrous hydrofluoric acid (HF) to cleave the benzyl ester linkage to the resin and thus to release the free polypeptide. The benzyl-derived side chain protecting groups are also removed by the HF treatment. The

polypeptide is then extracted from the resin, using 1.0 M acetic acid, followed by lyophilization of the extract.

Lyophilized crude polypeptides are purified by preparative high performance liquid chromatography (HPLC) by reverse phase technique on a C-18 column. A typical elution gradient is 0% to 60% acetonitrile with 0.1% TFA in H₂O. Absorbance of the eluant is monitored at 220 nm, and fractions are collected and lyophilized.

Characterization of the purified polypeptides is by amino acid analysis. The polypeptides are first hydrolyzed anaerobically for 24 hours at 110°C in 6 M HCl (constant boiling) or in 4 N methane sulfonic acid, when cysteine or tryptophan are present. The hydrolyzed amino acids are separated by ion exchange chromatography using a Beckman System 6300 amino acid analyzer, using citrate buffers supplied by Beckman. Quantitation is by absorbance at 440 and 570 nm, and comparison with standard curves. The polypeptides may be further characterized by sequence determination. This approach is especially useful for longer polypeptides, where amino acid composition data are inherently less informative. Sequence determination is carried out by sequential Edman degradation from the amino terminus, automated on a Model 470A gas-phase sequenator (Applied Biosystems, Inc.), by the methodology of R. M. Hewick et al., J. Biol. Chem., 256, 7990 (1981). Peptides could also be modified by amidation or various other means.

Polypeptide Carrier Conjugates

Polypeptides synthesized can be employed in the present invention in a monovalent state (i.e., free polypeptide or single polypeptide fragment coupled to a carrier molecule such as a biological carrier, including

collagen, a glycosaminoglycan or a proteoglycan, or albumin or the like). Preferably, as described below, to treat chronic inflammatory disorders, conjugates of multiple polypeptide fragments bound to a carrier molecule such as OA, HSA, other proteins, PEG, or the like are employed. Such modifications can increase the apparent affinity or change the circulatory half-life. The number of polypeptide fragments associated with or bound to each carrier molecule can be varied, but from about 4 to about 8 polypeptide fragments per carrier molecule are obtained under these coupling conditions.

Treatment of Inflammatory Disorders

As noted above, the polypeptides and their compositions modulate inflammation and are therefore useful in the treatment of a number of disease states in which aberrant inflammation plays a detrimental role. The method of the present invention is used to treat patients, most particularly humans afflicted with acute or chronic inflammatory disorders involving ischemia, infection, tissue swelling, and/or bone and cartilage degradation. Inflammatory disease refers to a condition in which activation of leukocytes leads to an impairment of normal physiologic function. Examples of such conditions include acute and chronic inflammation such as osteoarthritis, sepsis, ARDS (acute respiratory distress syndrome), immune and auto-immune disorders, rheumatoid arthritis, IBD (inflammatory bowel disease), lupus, MS, graft rejection, cirrhosis, sarcoidosis, granulomatous lesions, periodontitis/gingivitis, graft-vs.-host disease, contact dermatitis, and others. Other examples of such conditions include myocardial diseases, infectious diseases, and pulmonary diseases. Polypeptides corresponding to isolated fibronectin

residues can be used to treat inflammatory disorders. Although not necessary to practicing the invention, it is believed that immunosuppressive activity of fibronectin A chain-derived polypeptides block leukocyte
5 adhesion to endothelial cells and/or stromal/parenchymal and/or extracellular matrix, thus affecting leukocyte adhesion, cytokine production and recruitment at sites of inflammation. The method is particularly well suited for treating acute and/or chronic inflammatory
10 disorders, autoimmune disorders or disease conditions of the type described above.

Patient treatment using the method of the present invention involves administering therapeutic amounts of the polypeptide composition. In the context
15 of the present invention, the terms "treat" and "therapy" and the like refer to alleviate, slow the progression, prophylaxis, attenuation or cure of existing disease. A polypeptide composition may be formulated with conventional pharmaceutically acceptable
20 parenteral vehicles for administration by injection. These vehicles comprise substances which are essentially nontoxic and nontherapeutic such as water, saline, Ringer's solution, dextrose solution, Hank's solution, or the like. It is to be understood that polypeptide
25 formulations may also include small amounts of adjuvants such as buffers and preservatives to maintain isotonicity, physiological and pH stability. Preferably, the polypeptide or polypeptide carrier molecule conjugate is formulated in purified form
30 substantially free of aggregates and other protein at concentrations ranging from about 0.1 to about 10 mg/ml.

As indicated by the above formulation, the polypeptide may be administered parenterally. In the

case of some diseases, the polypeptide can be delivered or administered topically, by transdermal patches, intravenously, intraperitoneally, in aerosol form, orally, or in drops, among other methods. When the
5 polypeptide is administered intravenously, it can be delivered as a bolus or on a continuous basis.

The dose of the polypeptide formulation to be administered will depend upon the patient and the patient's medical history, and the severity of the
10 disease process. However, the dose should be sufficient to alleviate inflammation and tissue damage of the patient. Dosages for adult humans envisioned by the present invention and considered to be therapeutically effective will range from between about 10 and 100
15 mg/kg/day; however, lower and higher amounts could be more appropriate.

Animal Models of Inflammatory Diseases

The effectiveness of fibronectin polypeptides in the treatment of disease states in which aberrant
20 inflammation plays a detrimental role was evaluated using two representative animal models, SCW-induced arthritis and granuloma formation in Lewis rats and genetically constructed TGF- β 1-deficient mice exhibiting a wasting syndrome.

25 SCW-induced arthritis in Lewis rats closely mimics many features of human rheumatoid arthritis and is recognized as an animal model of arthritis in humans (see e.g., Wilder et al., Growth Factors, 2, 179-188 (1990); Wilder et al., J. Cell. Biochem., 45, 162-166
30 (1991); and Case et al., J. Clin. Investigation, Inc., 84, 1731-1740 (1989). Human rheumatoid arthritis and the animal model have a number of molecular and cellular aspects in common, including similarities in proteolytic

enzyme production and growth factor responsiveness. In both conditions, leukocyte activation and recruitment leads to inflammation and tissue swelling and/or tissue degradation.

- 5 TGF- β 1-deficient mice are created by genetic engineering and do not produce detectable amounts of either TGF- β 1 RNA or protein (see Kulkarni, Proc. Natl. Acad. Sci. USA, 90, 770-774 (1993)). Transforming growth factor betas (TGF- β s) are 25kd peptides produced
- 10 by virtually all cells of the body and exist in mammalian species as three isoforms, TGF- β 1, β 2, β 3. Of these, TGF- β 1 is the most widespread. There are other related molecules such as bone morphogenetic protein (BMP) of which there are a number of types; therefore
- 15 these represent an extended TGF- β family. TGF- β s are known to be intimately involved in many cellular processes such as cell proliferation and differentiation, embryonic development, extracellular matrix formation, bone development, wound healing,
- 20 hematopoiesis, and immune and inflammatory cell response. The modulation of immune and inflammatory responses by TGF- β s includes (i) the inhibition of proliferation of all T-cell subsets, (ii) inhibitory effects of proliferation and function of B lymphocytes,
- 25 (iii) down-regulation of natural killer cell activity and the T-cell response, (iv) regulation of cytokine production of immune cells, and (v) regulation of macrophage function (Kulkarni, Proc. Natl. Acad. Sci. USA, 90, 770 (1993)).
- 30 Two to three weeks after birth, TGF- β 1-deficient mice experience a wasting syndrome and the mice succumb shortly after becoming symptomatic. Histopathological analysis of the tissues from the symptomatic animals

reveals inflammatory lesions with massive infiltration of lymphocytes and macrophages in many organs and, particularly, in the heart and lungs. Many of the lesions resemble those found in autoimmune disorders, e.g., graft-vs.-host disease, and in certain viral diseases. The inflammatory lesions in the organs of TGF- β 1-deficient mice are associated with excessive leukocyte infiltration into the organs. The lungs typically exhibit a severe phlebitis with perivascular cuffing and a mixed lymphocyte/monocyte infiltrate. Within the heart, mononuclear phagocyte attachment and infiltration predominates and is associated with pathology in the endocardium, myocardium and pericardium.

The invention will be further described by reference to the following detailed examples.

EXAMPLES

Methods

20 Reagents

Fibronectin peptides were coupled to ovalbumin (OA; chicken egg, grade III, Sigman, St. Louis, MO) by dissolving equal amounts of lyophilized peptides (2-10 mg) and OA in a small volume of water (0.5-2 ml). In a second tube, ten times the amount of peptide of EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride was dissolved in water (300 μ l). The EDC solution was added to the peptide/OA mixture and rotated at 4 degrees for 2-18 hours. The mixture was then dialyzed into 4 L of PBS (Phosphate Buffered Saline, pH 7.4, NIH Media Unit), changing dialysis several times. Fibronectin polypeptide ovalbumin conjugates prepared contained about 4 to 5 polypeptide fragments per

ovalbumin molecule as determined by radiolabelling the peptide fragments prior to coupling and then evaluating the amount bound after coupling.

An additional study was performed in which the
5 FN peptide fragments were resuspended in PBS at a concentration of 2 mg/ml.

Animals - Lewis rats

Specific pathogen-free inbred Lewis (LEW)
10 female rats were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). The animals were approximately 100 g at the initiation of the experiments and were housed in ventilator filter units (Lab Products, Maywood, NJ). All injections were administered with
15 metophane anesthesia. Studies were performed following NIH-approved animal protocol.

Preparation of bacterial cell wall fragments

Group A streptococci from the American Type Culture Collection (SCW; ATCC 10389) were grown in Todd
20 Hewitt Broth (Difco, Detroit, MI), harvested in log phase, washed with PBS, incubated twice at 50°C with 4% sodium dodecyl sulfate (SDS), washed extensively to remove the SDS, and then incubated sequentially with DNase, RNase, and trypsin (4 hr at 37°C each; Sigma).
25 The washed cell walls were then sonicated for 70 minutes and the cell wall fragments remaining in the supernatant after 0.5 hr of centrifugation at 10,000 g were utilized for injection. The total amount of rhamnose in the cell wall-containing supernatant was determined by the
30 Dische-Shettles method, Dische and Shettles, J. Bio. Chem., 175, 595-603 (1948).

Induction, monitoring and treatment of arthritis

On day 0, each rat was injected intraperitoneally (i.p.) with an aqueous suspension of cell wall fragments containing 2.5 mg of rhamnose. In addition, each rat was injected intravenously (i.v.) with 1 mg of the coupled peptides in 0.5 ml PBS daily for five days. Control animals received an equal volume of PBS or OA. The rats were checked daily during the acute response and every other day thereafter. The severity of the arthritis manifested by each rat was determined using a "joint count" (Articular Index; AI). This score is derived by the summation of a score of 0 (normal) to 4 (maximum) for each extremity based on the degree of swelling, erythema, and distortion (maximum total score of 16). Additional studies examined the therapeutic efficacy of the peptides by administration on days 11-15, after the acute response had subsided, and at the initiation of the chronic phase.

In a parallel study, the efficacy of uncoupled FN peptides by i.v. administration in 0.5 ml PBS (1 mg) on days 0-4 was investigated.

Histologic Evaluation of Lewis rats

All rats were examined by routine histologic techniques. Joints were either fixed in 10% formalin, decalcified, sectioned, and stained with hematoxylin and eosin or quick-frozen in O.C.T. compound (Miles Scientific, Naperville, IL) by immersion in a mixture of dry ice and acetone, and sectioned for additional staining.

30

Animals - TGF- β 1-deficient Mice

Transforming growth factor β 1 null (TGF- β 1 (-/-)) mice were produced by targeted destruction of the

TGF- β 1 gene contained in a 5.7-kb *Bgl* II genomic fragment and transfection into mouse embryonic stem (ES) cells (Kulkarni et al., Proc. Natl. Acad. Sci. USA, 90, 770-774 (1993)). Mutated ES cells were injected into 3.5 day old blastocysts and transferred into the uterus of pseudopregnant mice (C57BL/6J) to produce chimeric heterozygous TGF- β 1 (+/-) mice. Chimeras were mated to produce offspring which were homozygous for the TGF- β 1 (-/-) gene mutation. Mice were housed in a double-barrier virus- and pathogen-free facility. Mouse genotype was verified by PCR analysis of extracted tail DNA (Kulkarni et al., Proc. Natl. Acad. Sci. USA, 90, 770-774 (1993)).

15

Spleen, Thymus and Blood Mononuclear Leukocytes
Isolation from TGF- β 1 (+/+), (+/-), and (-/-) Mice

TGF- β 1 (+/+), (+/-), and (-/-) mice (littermates) were sacrificed by CO₂ inhalation and lymphoid tissues were aseptically isolated. Thymus and spleen tissue specimens were pressed between sterile microscope slides (to prepare single cell suspensions), filtered through sterile 4x4 12 ply gauze (Johnson and Johnson Products Inc., New Brunswick, NJ) and centrifuged 1800 g, 10 min, 4°C). The resulting pellets were resuspended in 10 ml of ACK lysing buffer (B & B Research Laboratories, Fiskeville, RI) for 10 min at 4°C to lyse RBC, washed with PBS (1800 g, 10 min, 4°C), and resuspended in RPMI 1640 medium (containing heat inactivated 5% FBS, 2mM glutamine, 10 μ g/ml gentamycin, 50 μ M 2-mercaptoethanol) before being counted on a Coulter counter (Coulter Electronics Inc., Hialeah, FL).

Peripheral blood mononuclear leukocytes were isolated by ficoll (Histopaque; Sigma Chemical Co., St.

Louis, MO) centrifugation (900 g, 30 min, 23°C) of heparinized blood diluted in PBS.

Immunohistochemistry and Histological Evaluation

Selected tissues were placed in either
5 phosphate buffered saline (PBS), 10% neutral buffered formalin (10% paraformaldehyde in PBS), or 4% paraformaldehyde. Tissues in PBS were immediately embedded in Tissue-Tek O.C.T. Compound (Miles; Elkart, IN) and snap-frozen by immersion in liquid nitrogen or
10 in a dry ice-acetone bath. Snap-frozen tissues were stored at -70°C until used. Tissue samples fixed for at least 24 hr in 10% formalin were embedded in paraffin. Snap-frozen and paraffin-embedded tissues were sectioned (5 µM) and stained with hematoxylin and eosin for
15 histological analysis.

Endothelial Cell Culture

Mouse pulmonary artery (MPA) endothelia cells were generously provided by Dr. Una S. Ryan (Washington University, St. Louis, MO) and were sustained in culture
20 as previously described (Ryan and Maxwell, 1986, Biology of Endothelial cells). Cells were harvested mechanically for passage and subculture. Cells were seeded onto chambered slides at a concentration of 2.5×10^4 cells/ml. Adhesion assay slides were usually
25 prepared 24-48 hr (~70% confluent monolayer) prior to use.

Cell Attachment Assays

Cell attachment assays were performed using a
30 modification of a previously reported procedure (Wahl et al., Proc. Natl. Acad. Sci.: USA, 90, 4577 (1993)). Tissue culture chamber slides (8-well; Lab-tek, Nunc

Inc., Naperville, IL) were coated with purified fibronectin (33 kD fragment; 8 μ g/well), laminin, or pulmonary artery endothelial cells (-70% confluence). Human plasma fibronectin was purified by sequential ion-exchange and gelatin affinity chromatography, and the tryptic/catheptic 33kD heparin-binding fragment of the fibronectin A chain was isolated (McCarthy et al., Biochemistry, 27, 1380-1388 (1988)). To minimize non-specific binding of cells, bovine serum albumin (BSA; 1 mg/ml) was added to each well at 37°C for 1 hr and aspirated before cells were seeded in replicate wells at a density of 2.0 X 10⁶ cells/0.2 ml. After incubation for 30 min at 24°C or 37°C, the unattached cells were removed by two PBS washes, and the attached cells were fixed and stained with Diff-Quik (Baxter Scientific Products; McGraw Park, IL). Attached leukocytes were quantitated using the Optomax Image Analyzer (Hollis, NH) and the data expressed as the mean \pm SE. For inhibition assays, cells were incubated at 37°C for 15 min in the presence and absence of fibronectin polypeptides prior to being seeded in replicate wells coated with intact fibronectin 33 kD heparin-binding fragments.

25 Leukocyte Infiltration in Tissues of TGF- β 1 Deficient Mice

Although initially appearing normal, about 8 days post partum, mice homozygous for the TGF- β 1 mutation (-/-) began to exhibit leukocyte adherence to venules and tissue infiltration. Not all tissues are uniformly affected, but nearly 100% of the homozygotes exhibit both lung and cardiac pathology (Kulkarni et

al., Proc. Natl. Acad. Sci. USA, 90, 770-774 (1993)).
The lungs exhibit severe phlebitis with perivascular
cuffing and a mixed lymphocyte and monocyte
infiltration. Within the heart, mononuclear phagocyte
5 attachment and infiltration are predominately observed
and are associated with pathology in the endocardium,
myocardium and pericardium. Increased vessels in the
papillary myocardium, swollen infiltrated pericardium
and the continued accumulation of macrophages nearly
10 obliterated the myocardium contributing to the death of
the mice at 3-4 weeks of age.

Adhesion of Mononuclear Leukocytes to Extracellular
Matrix in vitro

15 In order to define the basis of the massive
leukocyte infiltration into the heart, lungs and other
tissues of the TGF- β 1-deficient mice, lymphoid cells
were isolated from (+/+) and (-/-) littermates and their
adherence properties compared in vitro. Mononuclear
20 leukocytes, whether derived from peripheral blood,
thymus or spleen of the (-/-) mice, were more adherent
to extracellular matrices than comparable populations
obtained from either (+/-) or (+/+) littermates. The
splenocytes derived from symptomatic 21 day-old TGF- β 1
25 (-/-) mice were -300% more adherent to an intact
fibronectin 33 kD heparin-binding fragments than
splenocytes from littermate controls. Similarly, TGF- β 1
(-/-)-derived mononuclear leukocytes adhered more
readily to laminin substrates than mononuclear
30 leukocytes from littermate controls.

Adhesion of Mononuclear Leukocytes to Endothelial
Cells in Culture

In parallel experiments, the interaction of

mononuclear leukocytes with endothelial cell monolayers was evaluated. A nearly 200% increase in the adherence of (-/-) splenocytes relative to that observed for either (+/+) or (+/-) was observed. These data suggest
5 that mononuclear leukocytes from TGF- β 1-deficient mice have enhanced adherence properties which likely contribute to the adhesion and migration of these cells into the tissues of the symptomatic animals.

10 Fibronectin Peptide Treatment of TGF β 1-deficient Mice

Four synthetic fibronectin polypeptides (I, V, CS-1 and MC-2) were evaluated for their ability to block
15 the adherence of TGF- β (-/-) leukocytes to fibronectin substrates and endothelial cell monolayers. The fibronectin polypeptides individually blocked leukocyte-fibronectin and leukocyte-endothelial cell adhesion. Based on these data, TGF- β 1 (-/-) mice were
20 injected with the active polypeptides in an effort to interrupt the widespread tissue infiltration and pathology. Since leukocyte adhesion to the vessel wall becomes evident on or around day 8 post partum, a mixture of the four fibronectin synthetic polypeptides
25 (I, V, CS-1 and MC-2) were administered intraperitoneally daily beginning on day 8 for 14-18 days. Confirmed TGF- β 1-deficient mice (by PCR analysis of tail DNA) were treated with a combination of the four fibronectin polypeptides at a total concentration of 4
30 mg/ml (prepared by dissolving 1 mg of each of the four polypeptides in 1 ml of solution). The mice (8 days-old) received a daily intraperitoneal injection (0.4 mg/0.1 ml) of the FN peptide cocktail. Even though asymptomatic, mice were documented to be TGF- β 1-
35 deficient mutants by PCR analysis of tail DNA. Animal weight as a marker of symptomology was monitored daily and as shown in Figure 4, peptide treatment was able to

retard the typical plateau and loss of weight evident in the untreated homozygote TGF- β 1-deficient animals. Three of three animals receiving this treatment showed diminished weight loss as well as a reduction in other
5 symptoms.

To evaluate the impact of the fibronectin polypeptides on the evolution of tissue pathology in these animals, the mice were sacrificed and tissues processed for light microscopic and ultrastructural
10 analysis. Three experiments were run and for each experiment all of the mice (untreated (-/-), FN treated (-/-) and wild-type (+/+) control) were sacrificed when the untreated TGF- β 1-deficient littermate (-/-) was close to succumbing. As represented by the heart
15 (Figure 5), but also evident in the lung (Figure 6), fibronectin polypeptide administration resulted in a virtual block of leukocyte infiltration into the tissue. Whereas the homozygous littermate (-/-) which received no polypeptides exhibited characteristic massive numbers
20 of inflammatory cells in the pericardium and myocardium (Figure 5B), the heart from the TGF- β 1-deficient littermate which was the recipient of daily intraperitoneal peptide therapy contained few, if any, inflammatory cells (Fig. 5C) and appeared more like
25 heart tissue of the normal wild-type (+/+) control (Figure 5A). This striking effect of the synthetic polypeptides on cardiac pathology was paralleled by reduced infiltration of leukocytes into the lung. (Compare the massive infiltration of inflammatory cells
30 observed in the lung tissue of untreated TGF- β 1-deficient mice (Figure 6B) with the lung tissue of the normal wild-type control (Figure 6A) and the TGF- β 1-deficient littermates which received daily i.p. fibronectin peptide therapy (Figure 6C)).

35

RESULTS

Effect of FN fragments on the development of SCW-induced arthritis

Daily i.v. administration of OA-coupled FN peptides on days 0-4 had an inhibitory effect on the evolution of arthritic lesions. The acute, neutrophil-mediated phase was blunted, but more dramatic was the suppression of the chronic, destructive phase (Fig. 1). On day 3, at the height of the acute response, the articular index (AI) was 8.5 ± 1.2 for the OA-treated, SCW-injected rats, which was reduced with treatment to 5.6 ± 0.8 with CS-1, 5.5 ± 0.84 with MC-2, 5.7 ± 1.1 with FNV, 6.6 ± 1.9 with FNII, and 2.0 ± 0.9 with FNI. Under these conditions, treatment with FNIV did not suppress the acute response, with an AI of 9.2 ± 0.97 .

By day 27, when chronic inflammation is well established, the differences were even more pronounced. The AI of the OA-treated, SCW-injected rats was 10.5 ± 0.3 . FNIV was the least effective in suppressing arthritis (AI = 8.6 ± 0.3). However, the other coupled peptides suppressed the AI to 2.5 ± 0.7 for CS-1, 1.25 ± 0.25 for MC-2, 1.7 ± 0.5 for FNV, 2.3 ± 0.9 for FNII and 1.5 ± 1.1 for FNI. No evidence of toxicity based on weight loss or hematocrit levels was observed in the peptide-treated groups.

Effect of FN fragments on the evolution of chronic arthritic lesions

To determine if the peptides could therapeutically suppress the chronic synovitis, administration was initiated after the acute response had fully developed. All the animals were randomized so that the AI for each group was similar, and peptide

administration was started on day 11 and continued daily until day 15 (5 days), well into the chronic phase. Surprisingly, all the FN peptides were suppressive (Fig. 2). On day 28, the OA-treated SCW-injected group had an AI of 11.3 ± 0.5 . Treatment with CS-1 reduced the AI to 4.2 ± 1.5 , MC-2 reduced it to 2.7 ± 1.9 , FNV to 4.5 ± 1.0 , FNIV to 2.0 ± 1.0 , FNII to 4.9 ± 1.2 , and FNI to 1.5 ± 1.0 . Based on these data, the primary target at this stage appears to be of leukocyte lineage (lymphocytes and/or macrophages) which are the central mediators of the chronic cell-mediated phase of arthritis in this model [Malone et al., J. Clin. Invest., 76: 1042-1056 (1985); and Wahl et al., J. Exp. Med., 168: 1403-1417 (1988)].

15

Effect of uncoupled FN fragments on the development of SCW-induced arthritis

Daily i.v. administration of uncoupled FN peptides on days 0-4 suggested an inhibitory effect on the development and maintenance of arthritis. In initial studies, the acute phase was slightly suppressed by all fragments (Fig. 3). On day 4, at the peak of the acute response, the AI of the untreated rats was 9.5, compared to a range of 3.3 to 6.7 after treatment with the FN fragments. Furthermore, the effect was sustained until day 24 when the AI of the untreated arthritic rats was 9.7, compared to 2.2 for the CS-1 treated animals, 1.3 after MC-2 treatment, 2.8 after FNV treatment, 1.0 after FNIV treatment, 1.7 after FNII treatment, and 5.0 after FNI treatment. These data show that the uncoupled FN fragments, in addition to multivalent FN peptides coupled to carriers or polymers (e.g., OA), are

effective in suppressing SCW-induced arthritis.

Effect of FN fragments on the histopathogenesis of SCW-induced arthritis

5 SCW induces synovial cell lining hyperplasia with villus formation, mononuclear cell infiltration, synovial proliferation, bone erosion, and ultimately, joint destruction which follows a pattern similar to human arthritis. Following peptide administration from
10 days 0 to 4, the joints exhibited markedly reduced histopathology when evaluated at the termination of the experiment. There was less infiltration of inflammatory cells, less synovial hyperplasia, and little evidence of erosions. In contrast, the OA-treated, SCW-injected
15 rats exhibited the destructive joint abnormalities characteristic of untreated groups of animals. Administration of the peptides during the early chronic phase of disease also effectively reduced the chronic, destructive pathology.

20

Effect of FN Fragments on the Wasting Syndrome of TGF- β -deficient Mice

The adhesion of leukocytes to the vascular endothelium is the earliest detectable event leading to
25 tissue pathology in the TGF- β 1-deficient mouse. Consistent with this adherence to the vessel wall in the target tissues, leukocytes isolated from the (-/-) homozygotes exhibit increased adherence to both extracellular matrix and to endothelial cell monolayers
30 in vitro. Synthetic FN polypeptides (I, V, CS-1 and MC-2) individually blocked the adhesion of TGF- β 1 (-/-) leukocytes to fibronectin substrates and endothelial cell monolayers in vitro.

Daily intraperitoneal administration of a mixture of four fibronectin polypeptides (I, V, CS-1 and MC-2) had an inhibitory effect on the infiltration of inflammatory cells into the heart and lung tissues of TGF- β 1-deficient mice. The fibronectin polypeptide treatment dramatically suppressed the weight loss exhibited by untreated control TGF- β 1-deficient mice at about day 17 (9 days after initiation of treatment; Figure 4). The weight gain and histopathology of the TGF- β 1-deficient mice treated with fibronectin polypeptides were very similar to that observed for the normal wild-type control (TGF- β 1 (+/+)). In some instances, histologic specimens of tissue from fibronectin polypeptide treated TGF- β 1 (-/-) mice could not be differentiated from wild-type mice, necessitating a repetition of the PCR analysis to characterize the genotype of the mice.

Based on the above example and written description, it has been shown that selected peptides derived from the extracellular matrix protein, fibronectin, are effective inhibitors of acute and chronic inflammatory pathology. Administration of fibronectin peptides with specific binding properties for integrins and cell surface proteoglycans (PG) or other CAMs can suppress joint diseases such as rheumatoid arthritis, acute or chronic inflammatory disorders, ischemia reperfusion and others.

The invention has been described with reference to various specific and preferred embodiments and techniques. It should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

SEQUENCE LISTING

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- (ii) TITLE OF INVENTION: Polypeptides Useful for Treating
Inflammatory Disorders
- (iii) NUMBER OF SEQUENCES: 6
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(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/139,903
(B) FILING DATE: 21-OCT-1993
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/990,296
(B) FILING DATE: 10-DEC-1992
- (viii) ATTORNEY/AGENT INFORMATION:
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(C) REFERENCE/DOCKET NUMBER: 600.283-US-01

(A) TELEPHONE: 612-332-5300
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(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Pro Gly Val

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 8 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Trp Gln Pro Pro Arg Ala Arg Ile
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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Pro Glu Ile Leu Asp Val Pro Ser Thr
20 25

(2) INFORMATION FOR SEQ ID NO:5:

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(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Pro Pro Arg Arg Ala Arg Val Thr
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ile Thr Val Tyr Ala Val Thr Gly Arg Gly Asp Ser Pro Ala Ser Ser
1 5 10 15

Lys Pro Ile Ser
20

WHAT IS CLAIMED IS:

1. A composition for use as a medicament for treating inflammatory disease in a mammal, said composition comprising a polypeptide having a sequence of at least about three amino acids corresponding substantially to an amino acid sequence within the 33 kD carboxyl terminal, heparin-binding region located on the A chain of fibronectin or an RGD-containing amino acid sequence within the 11.5 kD RGDS-mediated cell adhesion region located on all isoforms of fibronectin, and wherein said inflammatory disease includes any condition in which activation of leukocytes leads to an impairment of normal physiologic function.
2. The composition of claim 1 wherein said mammal is a human.
3. The composition of claim 1 wherein said inflammatory disease is an autoimmune disorder.
4. The composition of claim 1 wherein said inflammatory disease is an acute or chronic inflammatory disorder.
5. The composition of claim 1 wherein said inflammatory disease is arthritis.
6. The composition of claim 1 wherein said polypeptide has the formula: tyr-glu-lys-pro-gly-ser-pro-pro-arg-glu-val-val-pro-arg-pro-arg-pro-gly-val [SEQ ID NO:1].
7. The composition of claim 1 wherein said polypeptide has the formula: lys-asn-asn-gln-lys-ser-glu-pro-leu-ile-gly-arg-lys-lys-thr [SEQ ID NO:2].
8. The composition of claim 1 wherein said polypeptide has

the formula: trp-gln-pro-pro-arg-ala-arg-ile [SEQ ID NO:3].

9. The composition of claim 1 wherein said polypeptide has the formula: asp-glu-leu-pro-gln-leu-val-thr-leu-pro-his-pro-asn-leu-his-gly-pro-glu-ile-leu-asp-val-pro-ser-thr [SEQ ID NO:4].
10. The composition of claim 1 wherein said polypeptide has the formula: ser-pro-pro-arg-arg-ala-arg-val-thr [SEQ ID NO:5].
11. The composition of claim 1 wherein said polypeptide is employed as a conjugate having at least one polypeptide bound to a carrier molecule.
12. The composition of claim 11 wherein said carrier is ovalbumin.
13. A composition for use as a medicament for treating an autoimmune disorder in a mammal, said composition comprising a polypeptide/carrier molecule conjugate having at least three polypeptides bound to each carrier molecule of said conjugate, wherein said polypeptides suppress inflammation and the accompanying functional impairment in said mammal and are selected from the group of compounds having the formula: tyr-glu-lys-pro-gly-ser-pro-pro-arg-glu-val-val-pro-arg-pro-arg-pro-gly-val [SEQ ID NO:1], trp-gln-pro-pro-arg-ala-arg-ile [SEQ ID NO:3], asp-glu-leu-pro-gln-leu-val-thr-leu-pro-his-pro-asn-leu-his-gly-pro-glu-ile-leu-asp-val-pro-ser-thr [SEQ ID NO:4], ile-thr-val-tyr-ala-val-thr-gly-arg-gly-asp-ser-pro-ala-ser-ser-lys-pro-ile-ser [SEQ ID NO:6].

14. The composition of claim 13 wherein said carrier molecule is a biological carrier molecule.
15. The composition of claim 13 wherein said carrier molecule is ovalbumin.
16. The composition of claim 13 wherein said mammal is a human.
17. The composition of claim 13 wherein said autoimmune disorder is graft-vs.-host disease.
18. A composition for use as a medicament for treating an autoimmune disorder in a patient, said composition comprising a polypeptide of the formula: tyr-gly-lys-pro-gly-ser-pro-pro-arg-glu-val-val-pro-arg-pro-arg-pro-gly-val [SEQ ID NO:1], wherein said polypeptide suppresses inflammation and the accompanying functional impairment in said patient.
19. A composition for use as a medicament for treating an autoimmune disorder in a patient, said composition comprising a polypeptide of the formula: trp-gln-pro-pro-arg-ala-arg-ile [SEQ ID NO:3], wherein said polypeptide suppresses inflammation and the accompanying functional impairment in said patient.
20. A composition for use as a medicament for treating an autoimmune disorder in a patient, said composition comprising a polypeptide of the formula: asp-glu-leu-pro-gln-leu-val-thr-leu-pro-his-pro-asn-leu-his-gly-pro-glu-ile-leu-asp-val-pro-ser-thr [SEQ ID NO:4], wherein said polypeptide suppresses inflammation and the accompanying functional impairment in said patient.

21. A composition for use as a medicament for treating an autoimmune disorder in a patient, said composition comprising a polypeptide of the formula: ile-thr-val-tyr-ala-val-thr-gly-arg-gly-asp-ser-pro-ala-ser-ser-lys-pro-ile-ser [SEQ ID NO:6], wherein said polypeptide suppresses inflammation and the accompanying functional impairment in said patient.
22. A composition for use as a medicament for suppressing inflammation and the accompanying impairment of tissue function in a mammal, said composition comprising a polypeptide having a sequence of at least about three amino acids corresponding substantially to an amino acid sequence within the 33 kD carboxyl terminal, heparin-binding region located on the A chain of fibronectin.
23. A composition for use as a medicament for treating arthritis in a patient, said composition comprising a polypeptide/carrier molecule conjugate having at least three polypeptides bound to each carrier molecule of said conjugate, wherein said polypeptides suppress inflammation and the accompanying functional impairment in said mammal and are selected from the group of compounds having the formula: tyr-glu-lys-pro-gly-ser-pro-pro-arg-glu-val-val-pro-arg-pro-arg-pro-gly-val [sequence No. 1], lys-asn-asn-gln-lys-ser-glu-pro-leu-ile-gly-arg-lys-lys-thr [sequence No. 2], trp-gln-pro-pro-arg-ala-arg-ile [sequence No. 3], asp-glu-leu-pro-gln-leu-val-thr-leu-pro-his-pro-asn-leu-his-gly-pro-glu-ile-leu-asp-val-pro-ser-thr [sequence No. 4], ser-pro-pro-arg-arg-ala-arg-val-thr [sequence No. 5].
24. The composition of claim 13 wherein said carrier molecule is a biological carrier molecule.

25. A composition for use as a medicament for treating arthritis in a patient, said composition comprising a polypeptide of the formula: tyr-gly-lys-pro-gly-ser-pro-pro-arg-glu-val-val-pro-arg-pro-arg-pro-gly-val [sequence No. 1], wherein said polypeptide suppresses inflammation and functional impairment in said patient.
26. A composition for use as a medicament for treating arthritis in a patient, said composition comprising a polypeptide of the formula: lys-asn-asn-gln-lys-ser-glu-pro-leu-ile-gly-arg-lys-lys-thr [sequence No. 2], wherein said polypeptide suppresses inflammation and functional impairment in said patient.
27. A composition for use as a medicament for treating arthritis in a patient, said composition comprising a polypeptide of the formula: trp-gln-pro-pro-arg-ala-arg-ile [sequence No. 3], wherein said polypeptide suppresses inflammation and functional impairment in said patient.
28. A composition for use as a medicament for treating arthritis in a patient, said composition comprising a polypeptide of the formula: ile-thr-val-tyr-ala-val-thr-gly-arg-gly-asn-ser-pro-ala-ser-ser-lys-pro-ile-ser [sequence listing No. 6], wherein said polypeptide suppresses inflammation and functional impairment in said patient.
29. Use of a composition for the manufacture of a medicament for therapeutic use against inflammatory disease in a mammal, said composition comprising a polypeptide having a sequence of at least about three amino acids corresponding substantially to an amino acid sequence within the 33 kD carboxyl terminal,

heparin-binding region located on the A chain of fibronectin or an RGD-containing amino acid sequence within the 11.5 kD RGDS-mediated cell adhesion region located on all isoforms of fibronectin, and wherein said inflammatory disease includes any condition in which activation of leukocytes leads to an impairment of normal physiologic function.

30. Use of a composition for the manufacture of a medicament for treating an autoimmune disorder in a mammal, said composition comprising a polypeptide and carrier molecule conjugate having at least three of the following polypeptides bound to each carrier molecule of said conjugate, wherein said polypeptides suppress inflammation and the accompanying functional impairment in said mammal and are selected from the group of compounds having the formula: tyr-glu-lys-pro-gly-ser-pro-pro-arg-glu-val-val-pro-arg-pro-arg-pro-gly-val [SEQ ID NO:1], trp-gln-pro-pro-arg-ala-arg-ile [SEQ ID NO:3], asp-glu-leu-pro-gln-leu-val-thr-leu-pro-his-pro-asn-leu-his-gly-pro-glu-ile-leu-asp-val-pro-ser-thr [SEQ ID NO:4], ile-thr-val-tyr-ala-val-thr-gly-arg-gly-asp-ser-pro-ala-ser-ser-lys-pro-ile-ser [SEQ ID NO:6].
31. Use of a composition for the manufacture of a medicament for treating arthritis in a patient, said composition comprising a polypeptide/carrier molecule conjugate having at least three polypeptides bound to each carrier molecule of said conjugate, wherein said polypeptides suppress inflammation and the accompanying functional impairment in said mammal and are selected from the group of compounds having the formula: tyr-glu-lys-pro-gly-ser-pro-pro-arg-glu-val-val-pro-arg-pro-arg-pro-gly-val [sequence No. 1], lys-asn-asn-gln-lys-ser-glu-pro-leu-ile-gly-arg-lys-lys-thr [sequence

No. 2], trp-gln-pro-pro-arg-ala-arg-ile [sequence No. 3], asp-glu-leu-pro-gln-leu-val-thr-leu-pro-his-pro-asn-leu-his-gly-pro-glu-ile-leu-asp-val-pro-ser-thr [sequence No. 4], ser-pro-pro-arg-arg-ala-arg-val-thr [sequence No. 5].

32. Use of a composition for the manufacture of a medicament for suppressing inflammation and the accompanying impairment of tissue function in a mammal, said composition comprising a polypeptide having a sequence of at least about three amino acids corresponding substantially to an amino acid sequence within the 33 kD carboxyl terminal, heparin-binding region located on the A chain of fibronectin.
33. A method for treating inflammatory disease, which includes any condition in which activation of leukocytes leads to an impairment of normal physiologic function, in a mammal comprising: administering to said mammal an effective amount of a polypeptide having a sequence of at least about three amino acids corresponding substantially to an amino acid sequence within the 33 kD carboxyl terminal, heparin-binding region located on the A chain of fibronectin or an RGD-containing amino acid sequence within the 11.5 kD RGDS-mediated cell adhesion region located on all isoforms of fibronectin.
34. The method of claim 1 wherein said mammal is a human.
35. The method of claim 1 wherein said inflammatory disease is an autoimmune disorder.

36. The method of claim 1 wherein said inflammatory disease is an acute or chronic inflammatory disorder.
37. The method of claim 1 wherein said inflammatory disorder is arthritis.

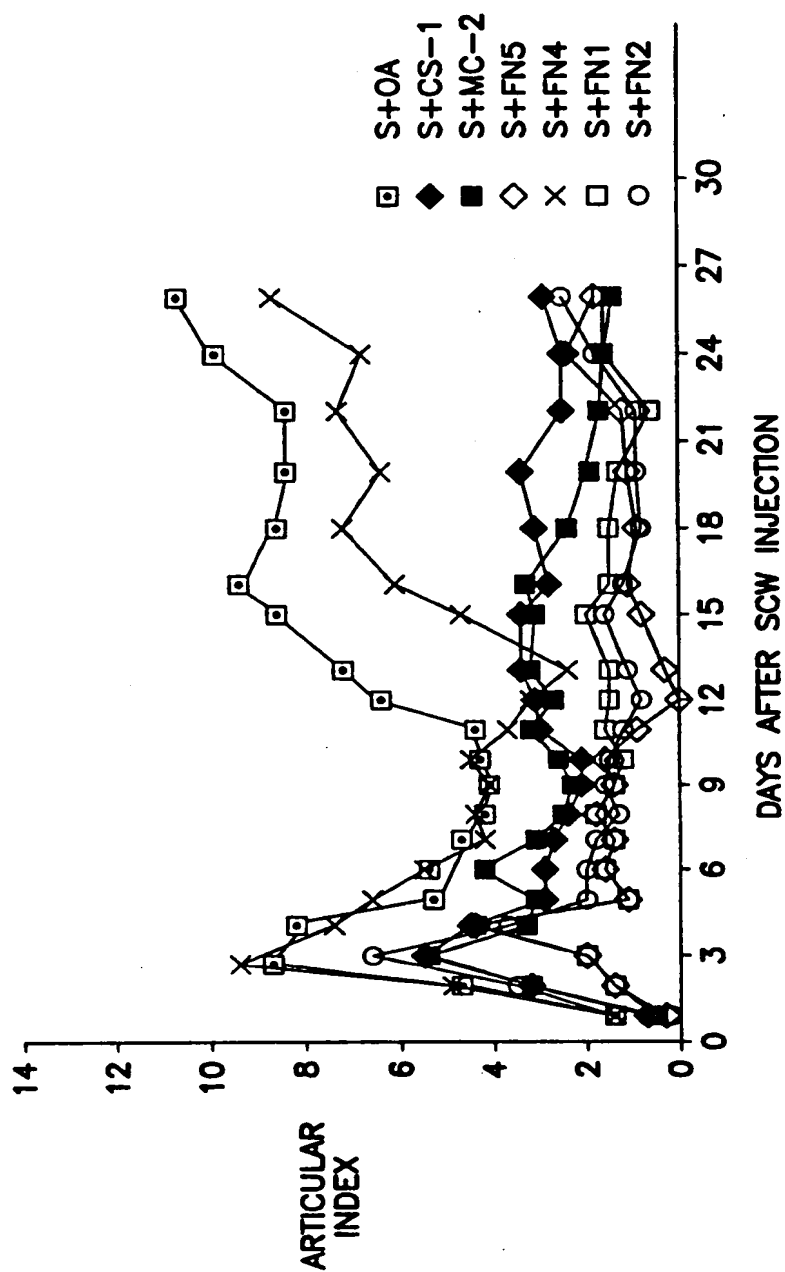


FIG. 1

2 / 5

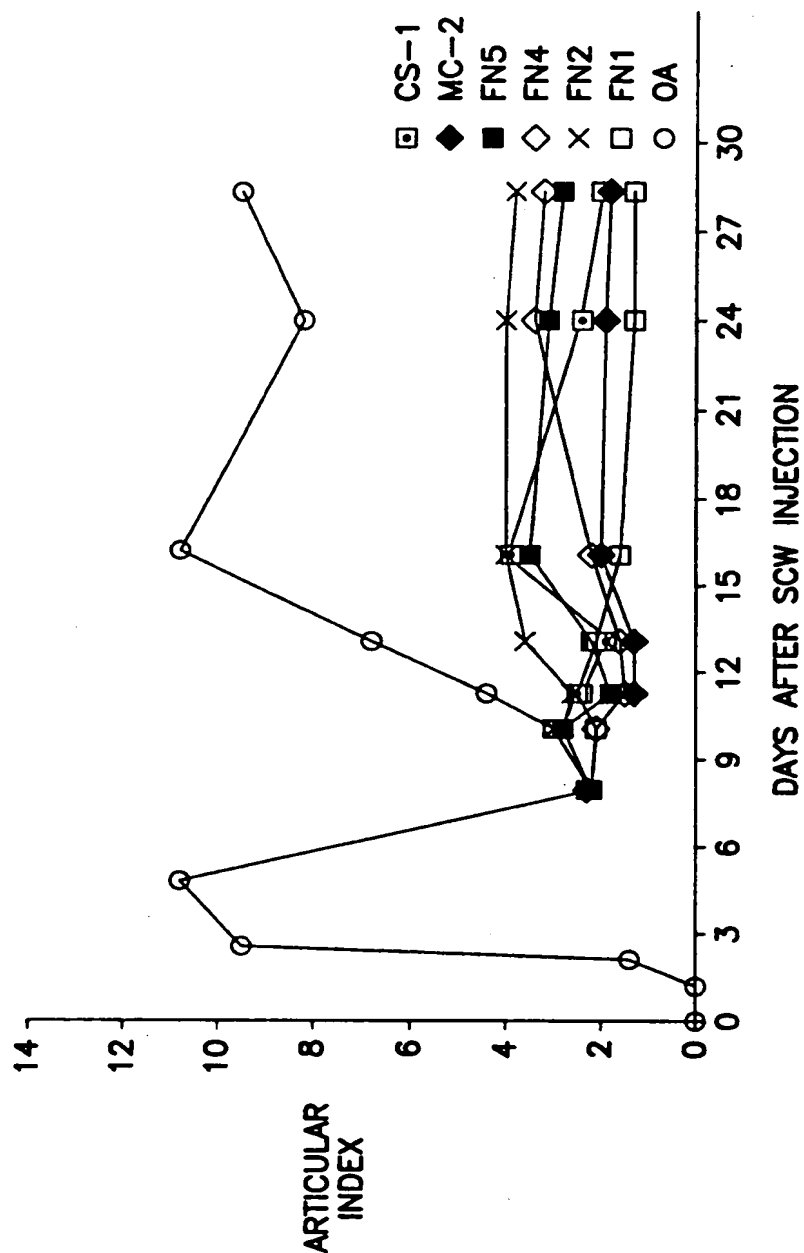


FIG. 2

3 / 5

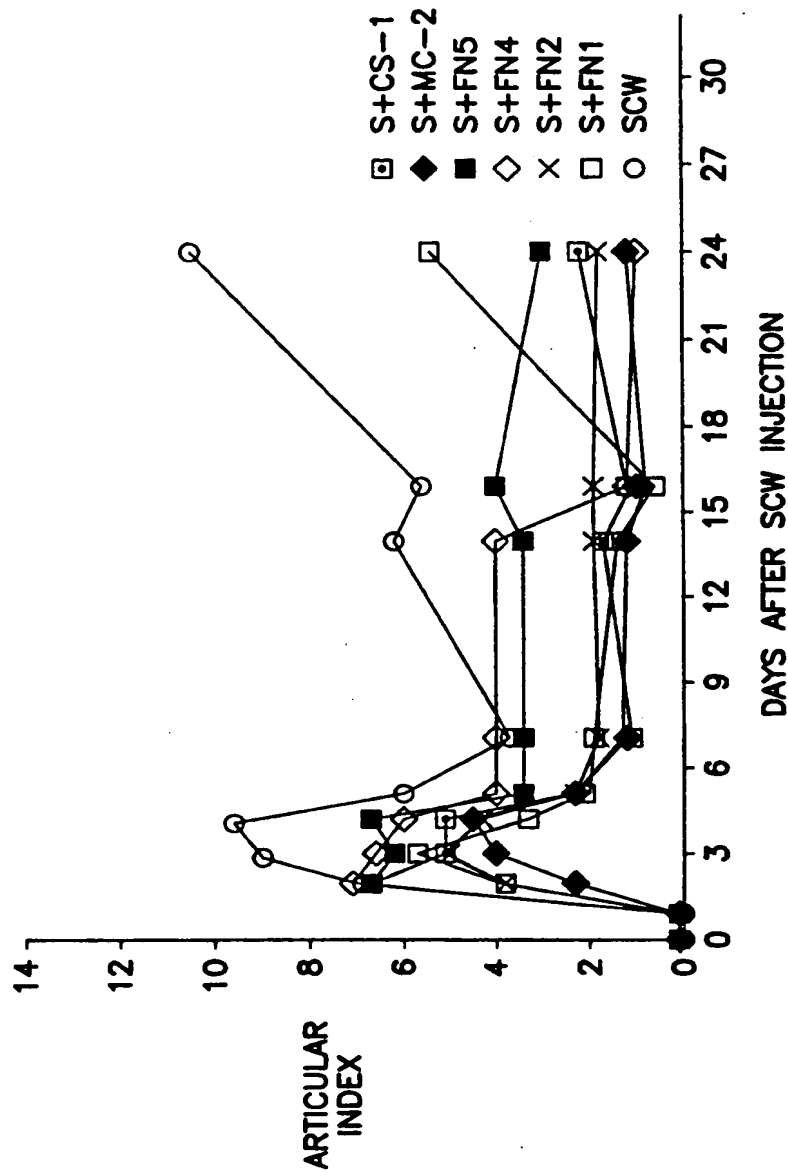


FIG. 3

4 / 5

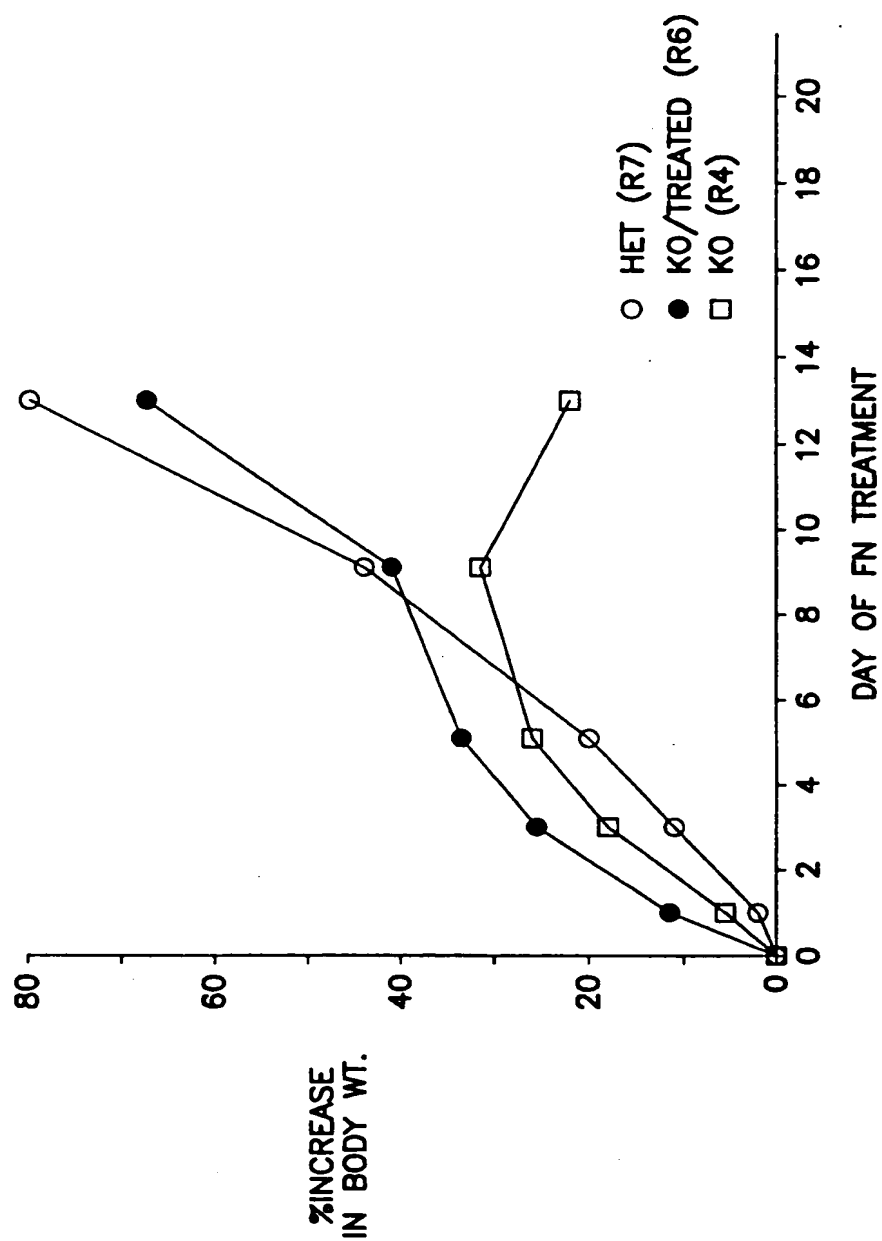
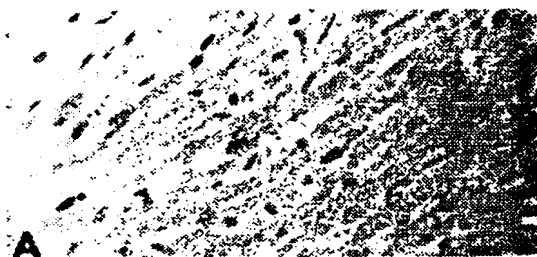


FIG. 4

SUBSTITUTE SHEET (RULE 26)

FIG. 5A



A

FIG. 5C

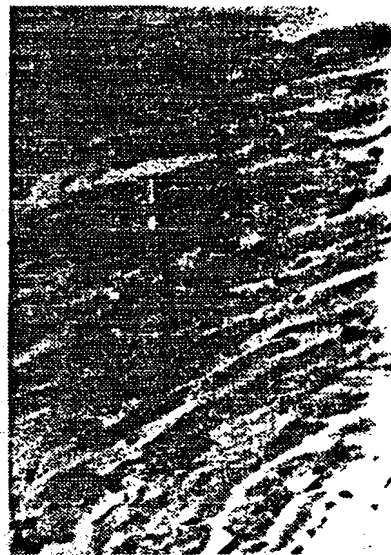
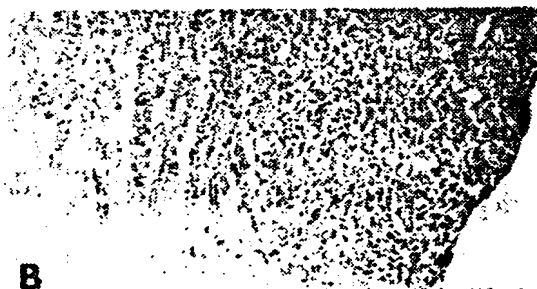
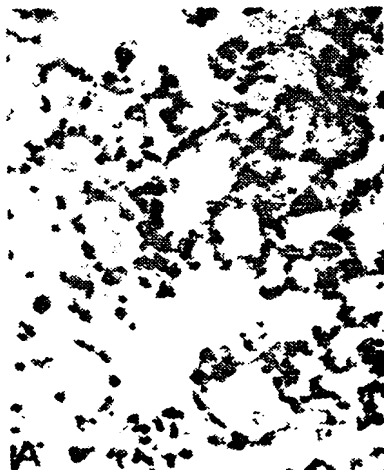


FIG. 5B



B

FIG. 6A



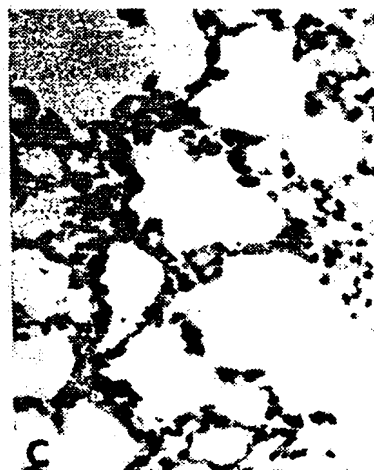
A

FIG. 6B



B

FIG. 6C



C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/11781

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C07K 5/00, 7/00, 7/04, 7/06, 7/08, 7/10; A61K 37/02

US CL : 514/13, 14, 15, 16, 17, 18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/13, 14, 15, 16, 17, 18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, MEDLINE, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US, 5,116,368 (McCARTHY et al.) 26 May 1992, see entire document.	1-10, 18-22, 25-29 and 32-37 ----- 11-17, 23-24, 30-31
X,P --- Y,P	US, 5,171,271 (FURCHT et al.) 15 December 1992, see entire document.	1-10, 18-22, 25-29 and 32-37 ----- 11-17, 23-24, 30-31

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, 3,966,902 (Chromecek) 29 June 1976, see entire document.	11-17, 23-24 and 30-31